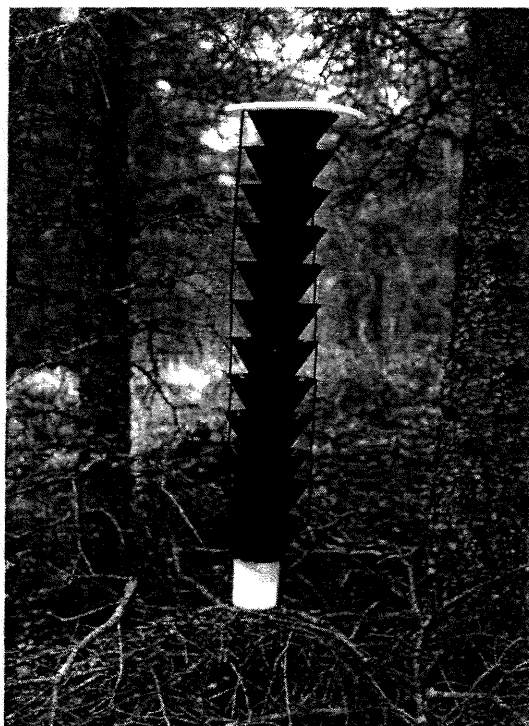


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Green Leaf Volatiles and MCH as Spruce Beetle Disruptants

An Exploratory Study



Technical Report R10-TP-92

August 2001


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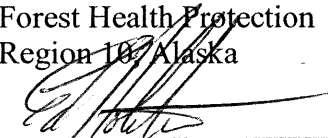
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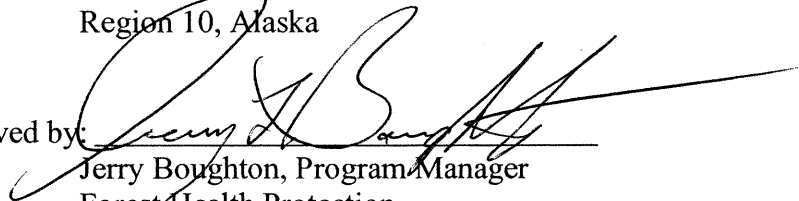
August 2001

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Abstract

Studies throughout the United States and Canada involving green leaf volatiles (GLV) have had success in disrupting bark beetle behavior. A recent study in British Columbia determined that two green leaf aldehydes, hexanal and (E)-2-hexanal, and one green leaf alcohol, hexanol, significantly reduced spruce beetle (*Dendroctonus rufipennis* (Kirby)) attraction to baited traps. Results from two GLV studies in south-central Alaska during 1997 and 1998 were inconclusive. In a 1999 Alaska study, however, hexanol and MCH (3-methylcyclohex-2-enone) significantly reduced spruce beetle attraction to funnel traps baited with semiochemicals. The MCH treatment alone out-performed all treatments.

Introduction

Spruce beetles (*Dendroctonus rufipennis* (Kirby)) are one of the most important, naturally occurring, mortality agents of Alaska's spruce forests. Endemic populations of spruce beetles breed in windthrown or felled trees. When spruce beetle populations increase and a sufficient supply of downed breeding material is no longer available for colonization, beetles may infest standing live trees, particularly in mature stands (Werner and others 1977). During the last decade, climatic, environmental and stand characteristics have been optimal for the spruce beetle development; in some years reducing the normal 2-year life cycle to one year, thus doubling beetle populations. Data gathered through insect and disease aerial detection surveys of Alaska indicate 2-3 million acres of white (*Picea glauca*), Lutz (*P. x lutzii*), and Sitka spruce (*P. sitchensis*) have been infested in the last ten years. In some areas the mortality has been so severe that 80-100% of the susceptible mature spruce has been killed (Wittwer 2000). This epidemic has affected a variety of resource objectives (visuals, wildlife habitat, fuels, fiber production, etc) across all land ownerships.

Research on pheromone isolation and identification has prompted much interest during the last decade in the application of pheromones and other semiochemicals to manipulate bark beetle populations (Salom and Hobson 1995, Shea 1994). Semiochemicals are compounds that can be detected by a diversity of organisms; pheromones are species specific semiochemicals. Field bioassays conducted from 1977 to 1979 in south-central and interior Alaska identified a two-component attractant (alpha-pinene and frontalin) for spruce beetles (Werner and Holsten 1995). Subsequent studies from 1988 to 1991 refined the release rate and enantiomers of the two-component lure and tested the addition of MCOL (1-methylcyclohex-2-enol) to the 2-component lure (Borden and others 1996, Werner 1994, Wieser and others 1989). MCOL greatly increased the

attractive properties of the lure but also increased the spill-over of beetles into surrounding live trees (Werner 1994).

The use of the antiaggregation pheromone for suppression of spruce beetle populations has had variable results (Zogas 2001). MCH (3-methyl-2-cyclohexen-1-one) is the primary antiaggregation pheromone naturally derived from female spruce beetles (Rudinsky 1973). MCH has been effective in laboratory and funnel trap tests but has yielded equivocal results in Alaska field tests for stand protection. Delivery methodology and elution rates of MCH in Alaska are difficult to control due to the cold climate.

Recent research has supported the hypothesis that combinations of semiochemicals can be more effective than a single disruptant such as an antiaggregation pheromone (Borden 1996). Green leaf volatiles (GLV's), esters commonly found in green plants, were found to repel conifer infesting bark beetles of the genera *Dendroctonus*, *Ips*, *Tomicus*, and *Hylurgops* (Dickens and others 1991, 1992 , 1993 and 1995, Schlyter and others 1998, Wilson and others 1996). Electroantennal studies of spruce beetles showed a response to 19 bark volatiles from black cottonwood (*Populus trichocarpa*) and paper birch (*Betula papyrifera*) (Huber and others 2000). Likewise GLV's reduced trap catches of the striped ambrosia beetle, *Trypodendron lineatum* (Borden and others 1997). A study in British Columbia tested the hypothesis that GLV's may disrupt the response of spruce beetles to attractant-baited traps. Two green leaf aldehydes, hexanal and (*E*)-2-hexenal, reduced the number of spruce beetles captured to intermediate levels and one green leaf alcohol, hexanol, significantly reduced spruce beetle trap catches (Poland and others 1998). Together, the green leaf alcohols and aldehydes reduced spruce beetle trap catches by 79 and 89 percent for males and females, respectively. In 1997 and 1998, GLV studies were conducted in south-central Alaska using the same aldehydes and alcohols as in Poland's 1998 study. Both studies did not result in significant differences between treatments due to: (1) low beetle populations in 1997 which did not provide enough "pressure" to

determine a treatment effect, and (2) in 1998: (a) the natural occurring pheromones from surrounding high spruce beetle populations were more attractive than the 2-component lure used in the funnel traps (eg. no significant difference between traps baited with the 2-component lure and unbaited traps); (b) a spill-over effect from nearby attacked trees affected trap catches; and (c) 25 percent of the traps were placed in a different stand composition which may have affected trap catches (Holsten and Matthews, data on file, Anchorage, AK).

Building on research results of Poland and others (1998) and preliminary Alaska GLV studies in 1997 and 1998, efforts to apply spruce beetle antiaggregants and GLV's were renewed in south-central Alaska. The following objective was developed and tested in 1999 through field studies: Determine the efficacy of various GLV's with and without MCH in reducing spruce beetle trap catches to attractant-baited funnel traps

Materials and Methods

Study Site:

The selected site, near the Granite Creek Campground, of the Chugach National Forest, on the Kenai Peninsula, in south-central Alaska, was a mixed stand of Lutz spruce and mountain hemlock (*Tsuga mertensiana*) with a small component of birch (*Betula papyrifera*). This cool site was located at approximately 650 feet msl with an elevation gradient of approximately 100 feet. Spruce beetle populations were slightly increasing (scattered single infested trees) in the study site.

Semiochemicals (Table 1) were dispensed from slow release devices (PheroTech, Inc., Delta, BC, Canada) placed in Lingren 12-funnel traps (Lindgren 1983). Traps were either suspended from spruce trees killed within the last two years or from non-host trees. Spruce beetles were collected

from the traps weekly from late May through the end of July. Trapped insects were placed in labeled plastic bags and frozen for later counting.

Experimental Design:

Treatments were replicated 10 times in a randomized complete block design. Traps were spaced about 100 feet apart. All possible combinations of GLV's and MCH were not possible due to the limited size of the study site. Treatments consisted of various semiochemicals with unbaited traps as controls:

1. Check (unbaited)
2. 3-component Spruce Beetle Lure only (α -pinene, frontalin, & MCOL)
3. 3-component Spruce Beetle Lure + hexenal + hexanol + MCH
4. 3-component Spruce Beetle Lure + hexanol + MCH
5. 3-component Spruce Beetle Lure + hexanol
6. 3-component Spruce Beetle Lure + MCH

Table 1. Release rates of synthetic semiochemicals used in *Dendroctonus rufipennis* trapping studies, Alaska, 1999^a.

Semiochemical	Amount	Release Rate (mg/day)	Dispenser
Frontalin	300 ul	2.6 @ 23°C	eppendorf vial
α -Pinene	600 ul	1.5 @ 20°C	eppendorf vial
MCOL	200 ul	2 @ 20°C	bubblecap
MCH	400 ul	4.5 @ 21°C	bubblecap
hexenal (AL)	650 ul	15 @ 22°C	bubblecap
1-hexanol (OL)	650 ul	4 @ 22°C	bubblecap

^a All semiochemicals have chemical purity greater than 98 percent.

Analyses:

All statistical analyses were completed using "Statistix 7" software. The Shapiro-Wilk Test was applied to the numbers of *D. rufipennis* caught by each treatment to determine whether data

conformed to a normal distribution. Since they did not, data were transformed using the natural log $X + 1$. Treatment effects were analyzed using ANOVA. Differences between means were tested using Tukey's (1953) comparison of the means test ($P = 0.05$). Only non-transformed means are reported in the results.

Results and Discussion

The spruce beetle 3-component lure-only treatment caught significantly higher numbers of beetles than the check treatment. The other four combinations tested were statistically the same as the check treatment (Table 2).

Table 2. Effect of GLV's and MCH on the response of *D. rufipennis* to the 3-component spruce beetle attractant released from Lindgren funnel traps, Granite Creek, Alaska, 1999.

TREATMENT	MEAN ¹ \pm SE	n ²
Check	1.6 ^b \pm 0.3	7
Spruce Beetle Lure Only (SB) ³	35.6 ^a \pm 16.7	7
SB + MCH	1.9 ^b \pm 0.8	7
SB + hexanol (OL)	8.2 ^b \pm 2.7	7
SB + OL + MCH	3.4 ^b \pm 1.7	7
SB + hexanal + OL + MCH	2.2 ^b \pm 1.6	7

¹Column values followed by the same letter are not significantly different ($P < 0.05$ Tukey's (1953) comparison of means test).

²Number of replications.

³alpha-pinene, frontalin and MCOL.

Two treatments, MCH and hexanol, each had a significant disruptive effect on the spruce beetle trap catches, 95% and 77% respectively. However, there was no significant treatment difference between hexanol with and without MCH. The addition of hexenal to the combination of hexenol and MCH did not significantly alter the disruptive effect when compared to the other treatment combinations. MCH was not included in Poland's 1998 study. It appears that GLV's do significantly affect trap catches. MCH, however, when used as the treatment, is as effective than either the GLV treatment

or a combination of MCH and GLV. MCH is readily available and relatively inexpensive as compared to either of the GLV's tested. On the other hand, the efficacy of MCH released from bubble caps and beads in Alaska field studies to deter spruce beetle attack have been less than stellar.

Acknowledgment

We thank John Hard, research entomologist (retired) Pacific Northwest Research Station, whose insight and assistance were extremely valuable and for his help in the installation of the trapping study. We also thank Karen Hyer, Pacific Northwest Research Station for statistical advice. We appreciate the reviews of Steve Munson, Skeeter Werner and Mark Schultz of earlier drafts of this paper.

Literature Cited

- Borden, J.H. 1996.** Disruption of semiochemical-mediated aggregation in bark beetles. pp. 421-438. In: Carde, R.T. and Minks, A.K. (eds.) *Pheromones Research: New Directions*. Chapman and Hall. New York.
- Borden, J.H.; Gries, G.; Chong, L.J.; Werner, R.A.; Holsten, E.H.; Wieser, H.; Dixon, E.A.; and H.F. Cerezke. 1996.** Regionally-specific bioactivity of two new pheromones for *Dendroctonus rufipennis* (Kirby) (Col., Scolytidae). *J. Appl. Entomol.* 120:321-326.
- Borden, J.H.; Chong, L.J.; Savoie, A.; Wilson, I.M. 1997.** Responses to green leaf volatiles in two biogeoclimatic zones by Striped Ambrosia Beetle, *Trypodendron lineatum*. *J. Chem. Ecol.* 23(11):2479-2491.
- Dickens, J.C.; Billings, R.R.; and Paynes, T.L. 1991.** Green leaf volatiles: a ubiquitous chemical signal modifies insect pheromone responses, pp. 277-280, In: Hrady (ed.). *Insect Chemical Ecology*. Academia Praha., Prague, Czechoslovakia,
- Dickens, J.C.; Billings, R.R.; and Paynes, T.L. 1992.** Green leaf volatiles interrupt aggregation pheromone response in bark beetles infesting southern pines. *Experientia* 48:523-524.
- Dickens, J.C.; Billings, R.R.; and Paynes, T.L. 1993.** Green leaf volatiles as inhibitors of bark beetle aggregation pheromones. United States patent No. 5,273,996.
- Dickens, J.C.; Billings, R.R.; and Paynes, T.L. 1995.** Green leaf volatiles as inhibitors of bark beetle aggregation pheromones. United States patent No. 5,468,770.
- Huber, D., Gries, R., Borden, J., Pierce Jr., H. 2000.** A survey of antennal responses by five species of coniferophagous bark beetles (Coleoptera: Scolytidae) to bark volatiles of six species of angiosperm trees. *Chemoecology*. 10:103-113
- Lindgren, B.S. 1983.** A multiple funnel trap for scolytid beetles (Coleoptera). *Can. Entomol.* 115:299-302.
- Poland, T.M.; Borden, J.H.; Stock, A.J.; and L.J. Chong. 1998.** Green leaf volatiles disrupt responses by the spruce beetle, *Dendroctonus rufipennis*, and the western pine beetle, *Dendroctonus brevicornis* (Coleoptera: Scolytidae) to attractant-baited traps. *J. Entomol. Soc. Brit. Columbia* 95:17-24.
- Rudinsky, J.A. 1973.** Multiple functions of the Douglas-fir beetle pheromone, 3-methyl-2-cyclohexen-1-one. *Environ. Entomol.* 2:579-585.
- Salom, S.M.; Hobson, K.R., tech eds. 1995.** Applications of semiochemicals for management of bark beetle infestations—proceedings of an informal conference. In: Annual meeting of the

Entomological Society of America. Gen. Tech. Rep. INT-GTR-318. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station.

Schlyter, Frederick; Löfqvist, Jan; Jakus, Rastislav. 1998. Green leaf volatiles and verbenone modify attraction of European *Tomicus*, *Hylurgops*, and *Ips* bark beetles. (Source unknown):29-44

Shea, P.J., tech. coord. 1994. Proceedings of the symposium on management of western bark beetles: research and development. Gen. Tech. Rep. PSW-GTR-150. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 53 p.

Tukey, J.W. 1953. The problem of multiple comparisons. Princeton, NJ: Princeton University. 396 p. Unpublished manuscript on file with with: Princeton University, Firestone Library, Princeton, NJ 08544.

Werner, R.A., Baker, B.H., and Rush, P.A. 1977. The spruce beetle in white spruce forests of Alaska. Gen. Tech. Rep. PNWGTR-61. U.S. Department of Agriculture, Forest Service, Pacific Northwest For. And Range Exp. Sta. 12 p.

Werner, R.A. 1994. Research on the use of semiochemicals to manage spruce beetles in Alaska, pp. 12-21. In: Shea, P.J. tech. coord. Proceedings of the symposium on management of western bark beetles with pheromones: research and development. 1992 June 22-25; Kailua-Kona, HI. Gen. Tech. Rep. PSW-GTR-150. Albany, CA. U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: 15-21.

Werner, R.A. and E.H. Holsten. 1995. Current status of research with the spruce beetle, *Dendroctonus rufipennis*. pp. 23-29. In: Salom, S.M.; Hobson, K.R., eds. Proceedings of the national Entomological Society of America meeting: application of semiochemicals for management of bark beetle infestations; 1993 Dec. 15; Indianapolis, IN. Gen. Tech. Rep. INT-GTR-318. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station: 23-29.

Wilson, I.M.; Borden, J.H.; Gries, R.; Gries, G. 1996. Green leaf volatiles as antiaggregants for the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Scolytidae). J. Chem. Ecol. 22(10):1861-1875.

Wittwer, D. 2000. Forest insect and disease conditions in Alaska—1999. Gen. Tech. Rep. R10-TP-82. Juneau, AK: U.S. Department of Agriculture, Forest Service, Alaska Region. 55 p.

Zogas, K.P. 2001. Summary of thirty years of field testing of MCH: Antiaggregation pheromone of the spruce bark beetle and the Douglas-fir beetle. U.S. Department of Agriculture, Forest Service, Alaska Region, Forest Health Protection Tech. Rep. R10-TP-91. 27 p.